

IN THE SPECIFICATION

Please substitute pages 25-34 with pages 25-29
submitted herewith.

Please add the Sequence Listing submitted herewith
as an attachment.



REMARKS

Applicants have amended the application by removing the Sequence Listing pages 25-30 and renumbering the subsequent pages accordingly. Applicants have further amended the application to add Sequence Listing pages 1-4 and a computer readable form thereof. Applicants have amended information in the numeric identifiers of the Sequence Listing. The amendments are marked in red on a copy of the original Sequence Listing as filed. These amendments include no new matter.

Respectfully submitted,

A handwritten signature in black ink, appearing to be "J. L." or similar, written over a horizontal line.

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June 22, 2001

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SEQUENCE LISTING

(1) ~~GENERAL INFORMATION:~~(i) ~~APPLICANT:~~

<110> Uwe Sonnenwald
 Marcus Ebnerth

- (A) NAME: IPK Gatersleben
 (B) STREET: Corrensstr. 3
 (C) CITY: Gatersleben
 (E) COUNTRY: Germany
 (F) ZIP CODE: 06466

<120>

(ii) ~~TITLE OF INVENTION:~~ 2-Deoxyglucose-6-phosphate (2-DOG-6-P)

phosphatase DNA sequences as selection marker in plants

<130> Vos - 12 GEN

<140> 09/558,284

(iii) ~~NUMBER OF SEQUENCES:~~ 4

<141> 2000-04-25

<150> PCT/EP98/02069

<151> 1998-04-09

(iv) ~~COMPUTER-READABLE FORM:~~

<160> 4

(A) ~~MEDIUM TYPE:~~ Floppy disk

(B) ~~COMPUTER:~~ IBM-PC-compatible

(C) ~~OPERATING SYSTEM:~~ PC-DOS/MS-DOS

<170> (D) ~~SOFTWARE:~~ Patent In Release #1.0, Version #1.30 (EPO)

<210>

(2) ~~INFORMATION FOR SEQ ID NO: 1:~~(i) ~~SEQUENCE CHARACTERISTICS:~~

<211>

(A) ~~LENGTH:~~ 758 base pairs

(B) ~~TYPE:~~ nucleotide <212> DNA

(C) ~~STRANDEDNESS:~~ single

(D) ~~TOPOLOGY:~~ linear

(ii) ~~MOLECULE TYPE:~~ cDNA(iii) ~~HYPOTHETICAL:~~ NO

(iv) ~~ANTI-SENSE~~: NO

(vi) ~~ORIGINAL SOURCE~~:

< 213 > (A) ~~ORGANISM~~: *Saccharomyces cerevisiae*

(ix) ~~FEATURE~~:

(A) ~~NAME/FEATURE~~: CDS

(B) ~~LOCATION~~: 9...746

< 400 >

(xi) ~~SEQUENCE DESCRIPTION~~: SEQ-ID-NO: 11

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Met Ala Glu Phe Ser Ala Asp Leu Cys Leu Phe Asp Leu Asp	
1 5 10	
GGT ACC ATA GTG AGT ACA ACA GTG GCC GCA GAG AAA GCA TGG ACC AAG	98
Gly Thr Ile Val Ser Thr Thr Val Ala Ala Glu Lys Ala Trp Thr Lys	
15 20 25 30	
TTG TGT TAC GAA TAC GGT GTT GAT CCT TCC GAG TTA TTT AAG CAT TCT	146
Leu Cys Tyr Glu Tyr Gly Val Asp Pro Ser Glu Leu Phe Lys His Ser	
35 40 45	
CAT GGT GCA AGA ACA CAA GAG GTT TTG AGA AGG TTT TTC CCT AAA TTG	194
His Gly Ala Arg Thr Gln Glu Val Leu Arg Arg Phe Phe Pro Lys Leu	
50 55 60	
GAT GAT ACA GAC AAT AAA GGT GTT CTT GCT CTA GAA AAA GAT ATT GCC	242
Asp Asp Thr Asp Asn Lys Gly Val Leu Ala Leu Glu Lys Asp Ile Ala	
65 70 75	
CAT AGT TAC TTG GAT ACA GTA AGC CTT ATT CCT GGT GCA GAG AAC TTA	290
His Ser Tyr Leu Asp Thr Val Ser Leu Ile Pro Gly Ala Glu Asn Leu	
80 85 90	
CTG TTA TCG TTA GAT GTA GAT ACT GAG ACT CAA AAA AAG TTA CCT GAA	338
Leu Leu Ser Leu Asp Val Asp Thr Glu Thr Gln Lys Lys Leu Pro Glu	
95 100 105 110	

AGG AAA TGG GCT ATC GTT ACC TCT GGT TCT CCA TAT TTG GCA TTT TCA	386
Arg Lys Trp Ala Ile Val Thr Ser Gly Ser Pro Tyr Leu Ala Phe Ser	
115 120 125	
 TGG TTC GAG ACA ATA TTG AAA AAT GTT GGA AAG CCC AAA GTT TTC ATT	434
Trp Phe Glu Thr Ile Leu Lys Asn Val Gly Lys Pro Lys Val Phe Ile	
130 135 140	
 ACT GGG TTT GAC GTG AAG AAC GGT AAG CCT GAT CCC GAG GGT TAT TCA	482
Thr Gly Phe Asp Val Lys Asn Gly Lys Pro Asp Pro Glu Gly Tyr Ser	
145 150 155	
 AGA GCT CGT GAT TTA TTG CGT CAA GAT TTG CAA TTA ACT GGT AAA CAG	530
Arg Ala Arg Asp Leu Leu Arg Gln Asp Leu Gln Leu Thr Gly Lys Gln	
160 165 170	
 GAT CTG AAG TAT GTT GTC TTC GAA GAT GCA CCC GTG GGC ATA AAG GCC	578
Asp Leu Lys Tyr Val Val Phe Glu Asp Ala Pro Val Gly Ile Lys Ala	
175 180 185 190	
 GGC AAA GCA ATG GGC GCC ATT ACT GTG GGT ATA ACA TCC TCG TAT GAC	626
Gly Lys Ala Met Gly Ala Ile Thr Val Gly Ile Thr Ser Ser Tyr Asp	
195 200 205	
 AAG AGC GTT TTA TTT GAC GCA GGA GCA GAT TAT GTA GTC TGT GAT TTG	674
Lys Ser Val Leu Phe Asp Ala Gly Ala Asp Tyr Val Val Cys Asp Leu	
210 215 220	
 ACA CAG GTT TCC GTG GTT AAG AAC AAT GAA AAC GGT ATT GTC ATC CAG	722
Thr Gln Val Ser Val Val Lys Asn Asn Glu Asn Gly Ile Val Ile Gln	
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240 245	

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(2) ~~INFORMATION FOR SEQ ID NO: 2;~~(i) ~~SEQUENCE CHARACTERISTICS:~~<211> (A) ~~LENGTH: 246 amino-acids~~(B) ~~TYPE: amino-acid~~ <212> PRT(D) ~~TOPOLOGY: linear~~ <213> *Saccharomyces cerevisiae*(ii) ~~MOLECULE TYPE: protein~~<400> (xi) ~~SEQUENCE DESCRIPTION: SEQ ID NO: 2a~~

Met Ala Glu Phe Ser Ala Asp Leu Cys Leu Phe Asp Leu Asp Gly Thr
 1 5 10 15

Ile Val Ser Thr Thr Val Ala Ala Glu Lys Ala Trp Thr Lys Leu Cys
 20 25 30

Tyr Glu Tyr Gly Val Asp Pro Ser Glu Leu Phe Lys His Ser His Gly
 35 40 45

Ala Arg Thr Gln Glu Val Leu Arg Arg Phe Phe Pro Lys Leu Asp Asp
 50 55 60

Thr Asp Asn Lys Gly Val Leu Ala Leu Glu Lys Asp Ile Ala His Ser
 65 70 75 80

Tyr Leu Asp Thr Val Ser Leu Ile Pro Gly Ala Glu Asn Leu Leu Leu
 85 90 95

Ser Leu Asp Val Asp Thr Glu Thr Gln Lys Lys Leu Pro Glu Arg Lys
 100 105 110

Trp Ala Ile Val Thr Ser Gly Ser Pro Tyr Leu Ala Phe Ser Trp Phe
 115 120 125

Glu Thr Ile Leu Lys Asn Val Gly Lys Pro Lys Val Phe Ile Thr Gly
 130 135 140

Phe Asp Val Lys Asn Gly Lys Pro Asp Pro Glu Gly Tyr Ser Arg Ala
 145 150 155 160

Arg Asp Leu Leu Arg Gln Asp Leu Gln Leu Thr Gly Lys Gln Asp Leu
 165 170 175

Lys Tyr Val Val Phe Glu Asp Ala Pro Val Gly Ile Lys Ala Gly Lys
 180 185 190

Ala Met Gly Ala Ile Thr Val Gly Ile Thr Ser Ser Tyr Asp Lys Ser
 195 200 205

Val Leu Phe Asp Ala Gly Ala Asp Tyr Val Val Cys Asp Leu Thr Gln
 210 215 220

Val Ser Val Val Lys Asn Asn Glu Asn Gly Ile Val Ile Gln Val Asn
 225 230 235 240

Asn Pro Leu Thr Arg Ala
 245

<210>

(2) ~~INFORMATION FOR SEQ ID NO: 3~~

(i) ~~SEQUENCE CHARACTERISTICS:-~~

<211>

(A) ~~LENGTH: 36 base pairs~~

(B) ~~TYPE: nucleotide~~ <212> DNA

(C) ~~STRANDEDNESS: single~~

(D) ~~TOPOLOGY: linear~~

(ii) ~~MOLECULE TYPE: other nucleic acid~~ <213> Artificial Sequence

(iii) ~~HYPOTHETICAL: YES~~ <220>

(iv) ~~ANTISENSE: NO~~

<223> Description of Artificial Sequence:
 Synthetic DNA sequence

<400>

(xi) ~~SEQUENCE DESCRIPTION: SEQ ID NO: 3~~

ATGGATCCCC ATGGCAGAAT TTTCAGCTGA TCTATG

< 210 >

(2) ~~INFORMATION FOR SEQ ID NO: 4:~~(i) ~~SEQUENCE CHARACTERISTICS:~~< 211 > (A) ~~LENGTH: 33 base pairs~~< 212 > (B) ~~TYPE: nucleotide DNA~~(C) ~~STRANDEDNESS: single~~(D) ~~TOPOLOGY: linear~~(ii) ~~MOLECULE TYPE: other nucleic acid~~ < 213 > Artificial Sequence(iii) ~~HYPOTHETICAL: YES~~ < 220 >(iv) ~~ANTISENSE: NO~~ < 223 > Description of Artificial Sequence:< 400 > (xi) ~~SEQUENCE DESCRIPTION: SEQ ID NO: 4;~~ Synthetic DNA Sequence

ATGTCGACTA CTCAGGCCCT TGTCAAAGGG TTG

Claims

1. Recombinant DNA molecule comprising
 - (a) regulatory sequences of a promoter active in plants;
 - (b) operably linked thereto a DNA sequence encoding a protein with the biological activity of a 2-deoxyglucose-6-phosphate (2-DOG-6-P) phosphatase; and
 - (c) operably linked thereto regulatory sequences which may serve as transcription termination and/or polyadenylation signals in plants.
2. The recombinant DNA molecule of claim 1, wherein the DNA sequence which encodes a protein with the biological activity of a 2-DOG-6-P phosphatase is selected from the group consisting of
 - (a) DNA sequences comprising a nucleotide sequence which encodes the amino acid sequence indicated in SEQ ID NO. 2;
 - (b) DNA sequences comprising the nucleotide sequence indicated in SEQ ID NO. 1;
 - (c) DNA sequences comprising a nucleotide sequence which hybridizes to a complementary strand of the nucleotide sequence of (a) or (b);
 - (d) DNA sequences comprising a nucleotide sequence which is degenerate to a nucleotide sequence of (c), and
 - (e) DNA sequences being a derivative, analogue or fragment of a nucleotide sequence of (a), (b), (c) or (d) and encoding a protein possessing 2-DOG-6-P phosphatase activity.
3. The recombinant DNA molecule of claim 1 or 2, wherein the DNA sequence is derived from yeast.

4. The recombinant DNA molecule of any one of claims 1 to 3, wherein the promoter is the 35S CaMV promoter.

5. Vector comprising a recombinant DNA molecule of any one of claims 1 to 4.

6. The vector of 5 which contains at least one further recombinant DNA molecule.

7. The vector of claim 6, wherein the further recombinant DNA molecule contains a DNA sequence which encodes a peptide, protein, antisense-, sense-RNA, viral RNA or ribozyme.

8. Host cell containing a recombinant DNA molecule of any one of claims 1 to 4 or a vector of any one of claims 5 to 7.

9. Kit comprising a recombinant DNA molecule of any one of claims 1 to 4 or a vector of any one of claims 5 to 7 and optionally 2-deoxyglucose or a chemical compound functionally equivalent to 2-deoxyglucose.

10. Process for selecting transformed plant cells, comprising the following steps:

- (a) obtaining plant cells;
- (b) introducing a recombinant DNA molecule of any one of claims 1 to 4 or a vector of any one of claims 5 to 7 into these plant cells; and;
- (c) selecting the successfully transformed plant cells on 2-deoxyglucose-containing media or on media containing a chemical compound which is functionally equivalent to 2-deoxyglucose.

11. The process of claim 10, wherein the vector of any one of claims 5 to 7 is transferred to plant cells via *Agrobacterium tumefaciens*.

12. The process of claim 10, wherein the recombinant DNA molecule of any one of claims 1 to 4 or the vector of any one of claims 5 to 7 is transferred to plant cells by particle bombardment.

13. Transgenic plant cell containing a recombinant DNA molecule of any one of claims 1 to 4 or a vector of any one of claims 5 to 7 or produced according to the process of any one of claims 10 to 12.

14. Plant cell of claim 13, which contains at least one further foreign gene.

15. Plant tissue comprising plant cells of claim 13 or 14 or produced according to the process of any one of claims 10 to 12.

16. Transgenic plant containing a plant cell of claim 13 or 14 or produced according to the process of any one of claims 10 to 12.

17. Harvest products of the plant of claim 16 comprising plant cells of claim 13 or 14.

18. Propagation material of the plants of claim 16 comprising plant cells of claim 13 or 14.

19. Use of a DNA molecule which comprises a DNA sequence as defined in any one of claims 1 to 3, of a recombinant DNA molecule of any one of claims 1 to 4 or

of a vector of any one of claims 5 to 7 for producing transgenic plants, plant cells and/or tissue.

20. Use of a DNA molecule which comprises a DNA sequence as defined in any one of claims 1 to 3, of a recombinant DNA molecule of any one of claims 1 to 4 or of a vector of any one of claims 5 to 7 as selectable marker in plant cell and tissue culture and/or plant breeding.

Abstract

Recombinant DNA molecules are described which contain a DNA sequence encoding a protein with the biological activity of a 2-deoxyglucose-6-phosphate
5 (2-DOG-6-P) phosphatase and being under the control of regulatory sequences of a promoter active in plants and transcription-termination and/or poladenylation signals. Also, vectors and hosts are described which contain the recombinant DNA molecules according to the invention.
10 Furthermore, processes for producing transformed plant cells and plants using the described recombinant DNA molecules and vectors are provided. The invention also describes transgenic plants, their harvest products and propagation material as well as plant cells and tissues
15 containing the recombinant DNA molecules or vectors according to the invention or having been produced by the process according to the invention.